214

EX-VIVO DETECTION OF CD8 T-CELL RESPONSES TO *IN-SILICO* PREDICTED HY MINOR HISTOCOMPATIBILITY ANTIGENS (mHAg) FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT)

Nakamura, R.[1], Tsai, W.[1], La Rosa, C.[2], Behrendt, C.[3], Lacey, S.[2], Srivastava, T.[2], Seidel, A.[2], Senitzer, D.[1], Forman, S.J.[1] [1] City of Hope National Medical Center, Duarte, CA; [2] City of Hope National Medical Center, Duarte, CA; [3] City of Hope National Medical Center, Duarte, CA

Minor histocompatibility antigens (mHAg) genes located on the Y chromosome (HY) differ substantially from homologs on the × chromosome (HX). Thus immunologic disparities occur in allogeneic HCT from female donor to male recipient (FtoM) and may explain the FtoM association with GVHD. Using *in-silico* prediction tools, we chose and synthesized 24 HY peptides (9-10mers) that bind HLA A0201 by T2 cell binding assay, yet differ in sequence from HX. With IRB approval, we collected one-time blood samples at 2 months-5 years post-HCT from adult male recipients. The primary group had HLA-A2+ female donor (n = 29); controls had HLA-A2+ male donor (n = 18) or nonHLA-A2+ female donor (n = 14). Additional controls were HLA-A2+ female nonpatients (n = 25); none was a donor. Expression of CD137 in CD8 T cells was measured by flow-cytometry after 24-hour stimulation of PBMC with one of 2 pools (I, II) of 12 HY peptides each. PBMCs exposed to media alone or CEF (CMV, EBV, influenza) peptides were negative and positive control, respectively. Response to HY peptide pool (minus response to media alone) was considered positive if above 0.07%. A majority of CD137+CD8+ cells had CD45RA+CD27- cytolytic effector profiles. CD8 responses to a few peptides were shared among multiple patients (i.e. LLLHCPSKTV derived from DFFRY, KLCKVRKITV from RPS4Y, and a previously described mHAg, FIDSYICQV, from SMCY). Positive response was more frequently observed in FtoM HLA-A2+ subjects than in controls combined, a finding more significant for responses to pool I (27.6% vs 7.0% +, $p < 0.01$) than to pool II (31.0% vs 15.8% +, $p = 0.10$). Agreement between categorical responses to pools I and II was 41.8% (95% CI 5.9%-77.8%) better than by chance among FtoM HLA-A2+ subjects but no better than chance in each control group. Continuous responses to pools I and II were not correlated in any group. FtoM HLA-A2+ recipients had 1.47-fold (95% CI 1.05-2.05) higher continuous response to pool I than did all controls combined. A similar association was not present for response to pool II. Independent effects of time since HCT, history of GVHD, current immunosuppressive therapy, and characteristics of donor and recipient on CD8 responses to HY peptides will be presented. In summary, novel HLA-A0201-restricted HY mHAg were predicted and validated in HCT patients. Our data may facilitate identification of clinically relevant immune targets of GVHD/GVL, and a biomarker for risk of GVHD based on CD8 responses to mHAg.

215

IMMUNE RECONSTITUTION AFTER DOUBLE UMBILICAL CORD BLOOD STEM CELL TRANSPLANTATION: COMPARISON WITH PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Jacobson, C.[1], Turki, A.[1], McDonough, S.[1], Stevenson, K.[1], Kim, H.[1], Herrera, M.[1], Reynolds, C.[1], Alyea, E.[1], Ho, V.[1], Koreth, J.[1], Soiffer, R.[1], Antin, J.[1], Ballen, K.[2], Cutler, C.[1], Ritz, J.[1] [1] Dana Farber Cancer Institute, Boston, MA; [2] Massachusetts General Hospital Cancer Center, Boston, MA

Patients who undergo umbilical cord blood (UCB) stem cell transplantation are at high risk of developing infectious complications. To define the immunologic deficiencies associated with UCB transplantation we compared immune reconstitution in patients who received either UCB stem cells (n = 42) or G-CSF mobilized blood stem cells from HLA-matched unrelated donors (MUD) (n = 102). All UCB recipients received 2 partially HLA-mismatched products; all patients received non-myeloablative conditioning. Blood samples were analyzed at 1, 3, 6, 9, 12, 18, and 24 months post-transplant for T, B, and NK cell surface markers, immunoglobulin levels, and B cell activating factor (BAFF) levels.

Reconstitution of CD3+ cells was significantly delayed in UCB compared to MUD for 1-6 months post-transplant ($p < 0.001$). This included naive (CD4+CD45RO-) and memory (CD4+CD45RO+) CD4 T cells, regulatory (CD4+CD25+) T cells, and CD8+ T cells. In contrast, CD19+ B cells recovered more rapidly in the UCB group and remained significantly greater from 3-24 months post-transplant ($p = 0.001$). CD56+CD16+ NK cells also recovered more rapidly in UCB patients and remained significantly greater from 1-24 months post-transplant. IgG and IgM levels recovered to normal range by 4-6 months, but IgA levels remained below normal during the follow-up period. BAFF levels were significantly higher in the UCB cohort at 1 month ($p < 0.001$) but were similar at 3 and 6 months. Despite similar BAFF levels, BAFF/CD19 B cell ratios were significantly lower in the UCB cohort at 3 ($p = 0.004$) and 6 months ($p = 0.005$).

This comparison of immune reconstitution after UCB and MUD transplantation in adults demonstrates that recovery of all T cells and T cell subsets is significantly delayed for a 6 month period while B and NK cell recovery is actually more rapid following UCB. UCB B cells appear to function normally resulting in early recovery of IgG and IgM levels. Higher BAFF levels early post-transplant in UCB patients may be responsible for the rapid and sustained B cell recovery but reasons for the delayed T cell recovery are not known. Early reconstitution of B cells, resolution of high BAFF levels, and reduced BAFF/B cell ratio in UCB patients who survive to T cell reconstitution may contribute to the reduced incidence of chronic GVHD ($p < 0.001$) in this group. Increased infectious complications following UCBT appear to be primarily due to selective global T cell deficiency in the first 6 months post-transplant.

216

LIVER IMMUNITY HAS PROTECTIVE FUNCTIONS AFTER HEMATOPOIETIC CELL TRANSPLANTATION BUT CAN CONTRIBUTE TO REJECTION OF THE GRAFT

Kuepper, N.J., Mueller, A.M.S., Tran, P., Shizuru, J.A. Stanford University, Stanford, CA

The liver is a critical immunocompetent organ that contains lymphocytes, natural killer (NK) cells, and antigen-presenting cells, including Kupffer cells, the resident macrophages. Because the liver is exposed to a variety of antigens as well as destructive and harmless toxins, it must provide immunogenic and tolerogenic immunity. Here, we studied the role of the liver on host protective immunity and donor engraftment post hematopoietic cell transplantation (HCT). Lethally irradiated BALB.b and BALB.k mice received MHC-matched FACS-purified hematopoietic stem cells (HSC) +/- splenocytes (SP) from C57BL/6 (B6, H2b) and AKR/J (H2k) donors, respectively. Frozen tissue and Ficoll-separated mononuclear cells (MNC) from livers, marrow, and blood were analyzed. Recipients of HSC+SP developed acute graft-versus-host disease (GVHD). The liver was a major target organ with massive donor T cell (TC) infiltration. However, lead-shielding of livers only during conditioning with total body irradiation completely protected mice from lethal GVHD. No infiltrating donor TC were detectable in unirradiated livers, however, donor engraftment was completely prevented. Examination of the marrows of these mice revealed donor cells comprised 3-10% of live cells on day 7 post-HCT, which were subsequently rejected. This observation implies that the liver contains immune cells that contribute to graft rejection. To test if this graft loss was due to alloreactivity GFP.B6 donor cells were infused into syngeneic B6 mice that were irradiated with/without liver shielding. Again, shielding of livers resulted in graft loss. In contrast, shielding of other body parts, such as legs, did not prevent donor cell engraftment. To clarify which cell populations mediate graft resistance, B- and TC deficient Rag2-KO and B-, T,- and NK cell deficient Rag2γc-KO mice were used as recipients. The median % of donor granulocytes in liver shielded Rag2- and Rag2c-KO was 16% and 31%, respectively, which is higher than in unirradiated, but significantly lower than in fully irradiated KO controls. The observation that lymphocyte deficient mice that received liver shielding could resist donor engraftment implies that non-lymphoid cells, i.e, those of the innate immune system play a central role in HSC resistance. In conclusion, our data highlight the importance of the liver in mediating engraftment of HSC, a feature that may often be overlooked as the liver is also major target of donor TC during acute GVHD.